

AMENDMENTS TO THE SPECIFICATION

On page 11, please replace the paragraph beginning on line 18 and ending on line 21 with the following amended paragraph:

Figure 2 shows the amino acid sequences of the peptide encoded by AtFtn2 (ARC6 gene) from a wild type plant in a WS ecotype (peptide of panel A, SEQ ID NO:2) and of the peptide encoded by arc6-1 gene in an arc6-1 mutant plant in a WS-like ecotype (peptide of panel B, SEQ ID NO:~~11~~ 206).

On page 12, please replace the paragraphs beginning on line 1 and ending on line 17 with the following amended paragraphs:

Figure 4 (SEQ ID NOS:86-114) shows a sequence alignment of DnaJ-like domains of plant and cyanobacterial Ftn2 proteins (indicated by asterisk) and DnaJ domains from Pfam database. Total about 270 DnaJ domains from the database were aligned with the ARC6 proteins. Shown in this figure are only selected DnaJ domains most similar to Ftn2 proteins. Black and gray columns indicate that identical or similar amino acid, respectively, was present in 70% of all aligned sequences at that position. The TrEMBL accession codes and location of the DnaJ domain within the protein are shown for the Pfam database records. For the ARC6 homologues, if the protein sequences were derived from EST records and did not encompass the initial M, the location of the DnaJ domain is not given.

Figure 5 (SEQ ID NOS:115-124) shows an alignment of plant and cyanobacterial Ftn2 full and partial sequences. Partial sequences are marked by asterisk (*). Not shown are the N-termini of the plant sequences, which contain chloroplast transit peptides. Light-gray and black columns indicate similarity and identity, respectively, greater than 80%. Gaps are indicated by a dash (-), missing sequence by an underline (_). Similarity and identity calculations do not include missing sequences. The Dna-J like domain is indicated by a solid line (—). Putative myb domain is indicated by diamonds (♦). Site of truncation of the protein in *arc6* mutant is marked by a triangle (▲) at position 398 of the alignment (residue 325 of AtFtn2).

On page 13, please replace the paragraphs beginning on line 15 and ending on line 23 with the following amended paragraphs:

Figure 25 (SEQ ID NOS:195-197) shows an alignment of the AtARC5 gene with Dynamin-1 from *Homo sapiens* and Dnm1p from *Saccharomyces cerevisiae*. Gray boxes indicate completely conserved residues; yellow boxes are identical residues; cyan boxes are similar residues; dashes indicate gaps. The domain structure is indicated by the lines above the alignment. Red, GTPase domain; green, middle domain; blue, PH domain; lavender, GTPase effector domain; black, PR domain. The dotted underline indicates the sequence encoded by the alternatively spliced intron in ARC5. The triangle indicates the position of the arc5 mutation.

Figure 26 (SEQ ID NOS:198-201) shows additional sequences which are homologous to AtARC5 gene.

Figure 27 (SEQ ID NOS:202-205) shows additional sequences which are homologous to AtFzo-like gene.

On page 40, please replace the paragraph beginning on line 24 and ending on page 41, line 3 with the following amended paragraph:

The product of the cyanobacterial *Ftn2* gene from *Synechococcus* sp. strain PCC 7942 was discovered to share a similarity with an unknown protein of *Arabidopsis thaliana* (AB016888|Q9FIG9; BLAST score, 72.8; Expect = 1 x 10⁻¹¹). It was therefore contemplated that this ortholog was involved in plastid division in *Arabidopsis* cells. The encoded product of this *Arabidopsis Ftn2* ortholog was predicted to posses a chloroplast transit peptide (from a web-based program (<http://HypothesisCreator.net/iPSORT/>), with the amino acid sequence MEALS HVGIG LSPFQ LCRLP PATTK LRRSH (SEQ ID NO:28). The *Arabidopsis* protein was also predicted to possess a DnaJ domain profile according to ProfileScan (http://www.isrec.isb-sib.ch/software/PFSCAN_form.html), and a Myb DNA-binding domain, according to InterProScan (<http://www.ebi.ac.uk/interpro/scan.html>).

On page 85, please replace the paragraph beginning on line 11 and ending on line 23 with the following amended paragraph:

Genomic DNA was isolated from WT and *arc6-11*, *arc6-2* and *arc6-3* young leaf tissue using the Plant DNAzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The *AtFtn2* genomic fragment was amplified with the *PfuTurbo* DNA polymerase (Stratagene, La Jolla, CA) using the primers 5' TGTCCAAATTTATGTGACACTCC 3' (forward) (SEQ ID NO:29) and 5' TTGTGAAAGGCTTGAATGTAAGA 3' (reverse) (SEQ ID NO:30). The amplification product of ~3.8 kb contained the whole *AtFtn2* coding sequence flanked by a 0.5 kb 5' and a 0.2 kb 3' regions. The amplified product was cloned into a *Sma*I-digested pBluescript vector (Startagene). For each plant genotype, DNA isolation, PCR amplification, and cloning of the product were carried out independently for three individual plants to minimize amplification errors. The resulting plasmid DNA was then pooled for each genotype and sequenced in both directions. Sequencer reads were processed, assembled into contigs, and viewed using Phrap, Phred and Consed (see the Software Tools section).

On page 100, please replace Table 6 with the following amended Table 6:

TABLE 6

DNA primers for PCR and sequencing of *Ftn2* and *Ftn6* of *Synechococcus* sp. PCC 7942

Primers	Used for PCR	Used for sequencing
<i>Ftn2</i> -specific		
Cpw267 5'-CCGAATTCTCTGTGTTGGCG-3' (D) (<u>SEQ ID NO:31</u>)	+	+
Cpw268 5'-AAGCTTCGTACAGACCCTGCTGAC-3' (R) (<u>SEQ ID NO:32</u>)	+	
Cpw338 5'-GGTAAGTTGACGGTCAAG-3' (D) (<u>SEQ ID NO:33</u>)	+	+
Cpw339 5'-CGATAGGGCCGTAGCTGTC-3' (R) (<u>SEQ ID NO:34</u>)	+	+
Cpw355 5'-GGTTAACCTGTGATCGAAC-3' (R) (<u>SEQ ID NO:35</u>)	+	+
Cpw376 5'-GCAGCCAGTCTGCCCTAG-3' (D) (<u>SEQ ID NO:36</u>)		+
Cpw377 5'-GCGCAGTCCTTCTTGAGG-3' (R) (<u>SEQ ID NO:37</u>)		+
Cpw384 5'-CTGACCGGTGAGGTTCTGC-3' (D) (<u>SEQ ID NO:38</u>)		+

Cpw386 5'- CCAGGAATCGCTAACATTC-3'(R) (<u>SEQ ID NO:39</u>)	+	
Cpw387 5'-GCGATCGCGTAGCTTCGG-3' (R) (<u>SEQ ID NO:40</u>)	+	
Cpw400 5'-CTAGGCAGTGTACGTTTC-3' (D) (<u>SEQ ID NO:41</u>)	+	
<i>Ftn6</i> -specific		
Cpw269 5'-CCGAATTCTGTGACCTCTACCCGTACTGC-3'(D) (<u>SEQ ID NO:42</u>)	+	+
Cpw270 5'-CCAAGCTTCGTTTATAAAGGCGCTCAG-3'(R) (<u>SEQ ID NO:43</u>)	+	+
Cpw340 5'-CTGCTCGTGAGCAATTGC-3' (D) (<u>SEQ ID NO:44</u>)	+	+
Cpw341 5'-CCGTTCTGAAAGGCTC-3' (R) (<u>SEQ ID NO:45</u>)	+	+
Cpw396 5'-CAGTGAATTGTAATAC-3' (D) (<u>SEQ ID NO:46</u>)		+
Cpw398 5'-GAAATAGCCATCGCGAGC-3'(R) (<u>SEQ ID NO:47</u>)		+

On page 101, please replace the paragraph beginning on line 2 and ending on line 13 with the following amended paragraph:

Orthologs *Ftn2*_A of *Ftn2* and *Ftn6*_A of *Ftn6* were identified in the genome of *Anabaena* sp. strain PCC 7120 by tblastn and blastn search against the complete *Anabaena* genome database at the Kazusa DNA Research Institute (kazusa.or.jp/cyano/anabaena). Copies of (i) *Ftn2*_A and (ii) *Ftn6*_A truncated at both ends were prepared by PCR with isolated genomic DNA of PCC 7120 as template using:

(i) CPW263, 5'-CCGAATTCTGTGGCAGTGGAAAATCGTGGG-3' (SEQ ID NO:48), as direct primer and CPW264, 5'-CCGAATTCCACTTGCACGATTGGGATC-3' (SEQ ID NO:49), as reverse primer and;

(ii) CPW265, 5'-CCGAATTGCCCTACTCATTAACATAG-3' (SEQ ID NO:50), as direct primer and CPW266, 5'-CCGAATTCCGGAGCGATCGCTTGTGTTG-3' (SEQ ID NO:51), as reverse primer. The PCR-generated copies were cloned in the *Eco*RI site of pRL498 (16), and the clones transferred by conjugation to wild-type PCC 7120, with selection on AA + nitrate agar medium (Fink A (1999) *Physiological Rev.* 79:6025-6032) containing 25 µg neomycin ml⁻¹.

On page 105, please replace the paragraph beginning on line 16 and ending on page 107, line 2 with the following amended paragraph:

The *arc5* mutation was previously mapped between markers nga 162 (20.6 cM) and AtDMC1 (32.6 cM) on chromosome 3 (Marrison et al., 1999 Plant J 18(6): 651-62). To fine-map the position of *arc5*, an F₂ population was generated from a cross between *arc5* and Col-0 wild type. 1720 mutant plants out of 7000 F₂ plants were selected and their DNA was extracted for PCR marker-based mapping. Markers were generated using the primer sets shown in Table 8:

Table 8
Primer Sequences

BAC Clone name	Primer sequences for PCR	Marker type
MDC8	GATTAATGAGACTATATGAGAG (<u>SEQ ID NO:52</u>) and ATCTGCATAACTCAATTGAACTG (<u>SEQ ID NO:53</u>)	INDEL
MCB22	GAACCCCCAGAATATCAACATC (<u>SEQ ID NO:54</u>) and GCTCTGATGGTGATTCTGGTAAC (<u>SEQ ID NO:55</u>)	INDEL
MVI11	GTAGCATTCTTAGAGATTGATCTAG (<u>SEQ ID NO:56</u>) and TATTCGAGTTGAAATTATGATTATGC (<u>SEQ ID NO:57</u>)	INDEL
MLD14	GCTACAGTTCTCAACCGGTAAATC (<u>SEQ ID NO:58</u>) and CATAAGCTTTATGCTCCAAAATAGTCTC (<u>SEQ ID NO:59</u>)	INDEL
T31J18	CTTGATCTGTGTTCTGACATCTC (<u>SEQ ID NO:60</u>) and CTAAACTATTACAAATGCCATAGACG (<u>SEQ ID NO:61</u>)	CAPS, cut by DraI
MMB12	AGCCGTCTTGTCCCATCATTAAG (<u>SEQ ID NO:62</u>) and GCACAAACAAACAGGGTCAATAGTTA (<u>SEQ ID NO:63</u>)	CAPS marker, cut by EcoRV

F16J14	TTAAAGTGAAGCTTAAGCAGAGG (<u>SEQ ID NO:64</u>) and CATTGTTAGAAAGTCAACACTTG (<u>SEQ ID NO:65</u>)	INDEL
MSA6	GCAAGACATAACCAATGAACAAG (<u>SEQ ID NO:66</u>) and GACACGTATGCGTTCTAAGAG (<u>SEQ ID NO:67</u>)	INDEL
MAL21	CTCCAACCTCAAGCAAAACGGATG (<u>SEQ ID NO:68</u>) and CTCTGTTTTGGGCTAGTGATGG (<u>SEQ ID NO:69</u>)	INDEL
MPN9	GCATACCCAATATCCTTGTC (<u>SEQ ID NO:70</u>) and GATAGTATAACCAGAGGTTGGAG (<u>SEQ ID NO:71</u>)	CAPS marker, cut by Tsp5091

The results indicated that *arc5* was located either on BAC clone MMB12 or MPN9, which overlap. The following three additional markers were generated, but no recombination between these and *arc5* was observed.

Table 9
Primer Sequences

BAC Clone name	Primer sequences for PCR	Marker type
MMB12	GAATCTTCTCAAAC TGAAATCCACC (<u>SEQ ID NO:72</u>) and TCGAAAGGAAGATCGGTGAACC (<u>SEQ ID NO:73</u>)	CAPS marker, cut by TaqI
MPN9	GATTGTGCTATGGTCAGGAGTTC (<u>SEQ ID NO:74</u>) and CATCAGCTATAACCTCCTCAGTG (<u>SEQ ID NO:75</u>)	CAPS marker, cut by AccI
MPN9	ACTGACTATAAGGACCCCTCAAAC (<u>SEQ ID NO:76</u>) and GTTGACCATAATTCCATCCACCACTATTA (<u>SEQ ID NO:77</u>)	INDEL but cut by HindIII

The mapping studies narrowed down the interval of chromosome III containing *arc5* to a 92-kb region comprising DNA spanning the overlap between MMB12 and MPN9.

On page 108, please replace the paragraph beginning on line 4 and ending on line 14 with the following amended paragraph:

To determine whether the wild type *ARC5* gene could complement the mutation, the predicted *ARC5* gene (a transgene containing the predicted At3g19730 /At3g19720 locus plus 1.9 kb and 1.1 kb of the 5' and 3' flanking DNA, respectively) was amplified from the DNA of BAC MMB12 by PCR using the primers 5'- GGAATTCCGAGTCGAGTTGCTTGTTG-3' (SEQ ID NO:78) and 5'- CGTCTAGAGCTTACCTCAAAGGTACATGGA-3' (SEQ ID NO:79). The PCR product was digested with *Eco*RI and ligated into a derivative of the transformation vector pLH7000 (http://www.dainet.de/baz/jb2000/jb_2000direkt.htm) digested with *Eco*RI and *Sma*I. The construct was transferred to *A. tumefaciens* GV3101 and introduced into *arc5* plants by floral dipping. The phenotypes of the T₁ plants were determined by microscopy. Microscopic analysis of T₁ transgenic plants indicated that the chloroplast division defect in the mutant was fully or partially rescued by the wild-type transgene.

On page 110, please replace the paragraph beginning on line 17 and ending on page 111, line 13 with the following amended paragraph:

The subcellular localization of *ARC5* was investigated by expressing a GFP-*ARC5* fusion protein in transgenic plants. The GFP sequence was amplified from plasmid smRS-GFP (Davis, S. J. & Vierstra, R. D. (1998) *Plant Mol. Biol.* 36, 521-528) with the primers 5'-CGGGATCCATGAGTAAAGGAGAAGAACT-3' (SEQ ID NO:80) and 5'-GCTCTAGATAGTTCATCCATGCCATGT-3' (SEQ ID NO:81). The PCR product was digested with *Bam*HI and *Xba*I. The *ARC5* coding region and 1.1 kb of the 3' flanking DNA were amplified from the MMB12 BAC clone with primers 5'-GGACTAGTACGATGGCGGAAGTATCAGC-3' (SEQ ID NO:82) and 5'-CGGGATCCGCACCGAAGGAGCCTTAGATT-3' (SEQ ID NO:83). The PCR product was digested with *Spe*I and *Eco*RI. cDNA fragments encoding GFP and *ARC5* were subcloned into Bluescript KS+ (Stratagene) that had been digested with *Eco*RI and *Bam*HI to

create a *GFP-ARC5* fusion construct. The *ARC5* promoter was amplified from MMB12 with primers 5'-GACTAGTTGGCTAACGCTTACCTCAA-3' (SEQ ID NO:84) and 5'-CGGGATCCGCCATCGTCTCTTACGA-3' (SEQ ID NO:85), and cloned into Bluescript KS+ (Stratagene) between the *SpeI* and *BamHI* sites. The promoter fragment was then subcloned into the plasmid containing the *GFP-ARC5* fusion construct at the 5' end of the fusion. The resulting plasmid was digested with *SpeI* and *EcoRI*, and the promoter-*GFP-ARC5* cassette was subcloned into a derivative of the transformation vector pLH7000 (http://www.dainet.de/baz/jb2000/jb_2000direkt.htm). The plasmid was transferred to *A. tumefaciens* GV3101 and used to transform wild-type *A. thaliana* plants (Col-0) as described above. The *GFP-ARC5* localization-pattern was visualized by fluorescence microscopy in T₁ plants. For *in vivo* detection of green fluorescent protein (GFP), fresh leaf tissue was mounted in water and viewed with an L5 filter set (excitation 455 nm to 495 nm, emission 512 to 575 nm) and a 100X oil immersion objective of a Leica DMR A2 microscope (Leica Microsystems, Wetzlar, Germany) equipped with epifluorescence illumination. Images were captured with a cooled CCD camera (Retiga 1350EX, Qimaging, Burnaby, British Columbia, Canada) and processed with Adobe Photoshop imaging software (Adobe Systems, San Jose, CA).

Please insert the attached Sequence Listing as new pages --116--333--.

IN THE CLAIMS

Please renumber the Claims pages from pages "116-120" to --334-338--.

IN THE ABSTRACT:

Please renumber the Abstract page from page "121" to --339--.

AMENDMENTS TO THE DRAWINGS

Please replace Figures 1 and 8 with the substitute Figures 1 and 8 attached hereto. In addition, Applicants are providing the Office with a complete set of drawings (Figures 1-27) having proper margin sizes as requested in the Notice to File Missing Parts.